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THE INFLUENCE OF GLYCEMIC CONTROL ON HEART RATE VARIABILITY AND BLOOD PRESSURE IN ADULT PATIENTS WITH THE TYPE 1 DIABETES

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Abstract

Background and aims*: to identify heart rate variability (HRV) and blood pressure (BP) in patients with type 1 diabetes depending on the duration of disease and glycemic control.* ***Materials and methods:*** *43 patients were examined. All patients were divided into 2 groups according to the level of HвA1c: group 1 (n=21) with HвA1c ≤ 7.5% and group 2 (n=22) with HвA1c > of 7.5%. All patients underwent daily monitoring of electrocardiogram Holter and ambulatory BP monitoring within 24 hours in parallel with long term monitoring of blood glucose.* ***Results:*** *Hyppoglycemia is characterized by significant decrease root mean square difference between adjacent RR intervals (RMSSD) (r = - 0.531; p = 0.003) and number of consecutive RR intervals, the difference between them is more than 50 ms expressed as a percentage of total number of RR-intervals (pNN50%) (r = the - 0.503; p = 0.005) and increase of Low Frequency/High Frequency Ratio (LF/HF) (r = 0552; p = 0.002). Patients with hypoglycemia had significantly higher daily diastolic pressure area index (DPAI24) (p = 0.016), and daily diastolic pressure time index DPTI24 (p = 0.025).* ***Conclusion:*** *our findings demonstrate the need to reduce the frequency of hypoglycemia episodes in patients with T1DM.*

key words: *diabetes, hypoglycemia, heart rate variability*

Background and aims

According to International Diabetes Federation (IDF) 415 million adult people suffer from diabetes mellitus (DM) in the world. The number of cases will reach 642 million people by 2040 with preserving actual trends. Type 1 diabetes mellitus (Type 1 DM) will raise from 7% to 17%. Although Type 1 DM is less common than Type 2 DM, the annual growth is approximately 3% in the world [1].

Type 1 DM leads to disability in young and middle age, due to progression of its complications (mainly nephropathy and amputations) [2-4]. Evolving metabolic changes contribute to the development of cardiovascular complications in the patients' employable category that is results both in medical and social problems. Mortality from cardiovascular disease in patients with diabetes is 2-3 times higher than among others [4-6].

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The clinical manifestations of the cardiovascular system involvement in Type 1 DM include resting tachycardia, accompanied by lengthening of the QT interval, the fixed heart rate (syndrome denervation of the heart), and reduced tolerance to physical activity. Also, orthostatic hypotension, and painless myocardial ischaemia are included according to the recommendations of the American Diabetes Association (ADA) [7].

Lengthened QT interval leads to increased risk of arrhythmias [8,9]. Ventricular arrhythmias are especially dangerous. They can lead to sudden death due to acute heart failure – the so-called "dead-in-bed" syndrome [10,11]. Firstly, the clinical value of analysis of heart rate variability (HRV) was revealed in the early 60`. Under physiological conditions, regulation of heart rhythm is the result of automatic cells' rhythmic activity in the sinus node, which modulates the influence of the autonomic and central nervous systems, humoral and reflex effects. The decrease of HRV is regarded as a powerful predictor of sudden cardiac death in patients with cardiovascular disease, as well as among others. In addition, the decrease of HRV is closely associated with the deterioration of the functional state of the cardiovascular system [12]. Technologies were standardized and recommendations for practical use of HRV indexes were developed by the working group of the European society of cardiology and the North American society of pacing and electrophysiology in 1996 [13,14].

Nowadays, the unsolved problem of occurrence of hypoglycemic conditions in patients with type 1 diabetes remains. They exert influence on pro-arrhythmogenic action and accelerate the development of both micro - and macrovascular complications of diabetes, despite the new types of insulin.

The aim of the investigation was to reveal changes of heart's rate variability and blood pressure in patients with type 1 diabetes depending on disease's duration and glycemic control.

Materials and methods:

Fourty-three patients with DM (males-23 (53%), females - 20 (47%) were examined in the Endocrinology Department of University Clinic of «Dnepropetrovsk Medical Academy» between October 2016 and September 2017.

The diagnosis of diabetes mellitus type 1 was made according to American Diabetes Association criteria - 2017.

The inclusion criteria were: males and females with type 1 diabetes, from 18 to 40 years old without arterial hypertension.

The exclusion criteria were: type 2 diabetes; diabetic ketoacidosis at the time of inclusion; secondary DM; body mass index (BMI) > 40 kg/m2; diabetic proliferative retinopathy; chronic kidney diesease IIIB - V; diabetic foot (class II according to Wagner and above); heart failure III / IV by the *New York Heart Association* (*NYHA*); congenital and acquired heart disease; acute coronary syndrome, acute ischemic stroke and transient ischemic attack; exacerbation of accompanying chronic diseases; acute physical illness; myocarditis in past history; cancer up to 5 years from the full course of therapy; antiretroviral therapy; diagnosed viral hepatitis B and C; pregnancy.

All patients had signed the voluntary informed consent, approved by the local Ethics Committee before their inclusion in the study. All patients were treated on the basal-bolus insulinotherapy, the daily dose of insulin was 44 ± 3,15 IU. The C-peptide, HbA1c, blood creatinine, the first morning urinary albumin excretion (UAE) were determined for verification and evaluation of control. C-peptide

was determined on an electrochemiluminescence automatic immunochemical analyzer COBAS e 411, Roche Diagnostics GmbH & Hitachi, Japan, 2012. The concentration of blood creatinine, HbA1c and microalbumin were determined using automatic biochemical analyzer SAPPHIRE 400, TokioBoeki, Japan, 2009. GFR was calculated according to the formula CKD-EPI [15].

Long-term monitoring of blood glucose levels was conducted by using the system CGMS (Continuous Glucose Monitoring System, Medtronic MiniMed, USA).

This system detects electrical signals every 10 seconds and transforming them in glucose values every 5 minutes. The boundaries of the measurement of glucose levels by this method range from 2.2 mmol/l to 22.2 mmol/l [16]. The patients performed at least four blood glucose measurements per day using the individual glucose test for the calibration data of the sensor with capillary blood. Hypoglycemia was considered as an episode of lower blood glucose level less than 3.9 mmol/l according to the criteria of ADA [7].

The daily monitoring of the electrocardiogram (ECG) Holter and extended monitoring of blood pressure (BP) within 24 hours on the unit DigiTrak XT (Philips Medical Systems, USA) were carried out in the free moving regime. It was made in parallel with long term monitoring of blood glucose. The temporal and spectral HRV parameters were examined, which were computed using the software ARNIKA, version 8.3.9.

Analysis of the time domain was carried out using these indicators:

SDNN (ms) – the standard deviation of normal intervals RR (NN), which represents the total effect of vegetative regulation of the heart.

RMSSD (ms) – the root mean square difference between adjacent RR intervals. It reflects the activity of parasympathetic link of vegetative regulation.

PNN50 (%) - number of consecutive RR intervals, the difference between them is more than 50 ms expressed as a percentage of the total number of RR-intervals, reflecting the degree of predominance of parasympathetic link of regulation over the sympathetic (relative value).

The circadian profile was assessed by the circadian index (CI), which was calculated as the ratio of the average daily to the average night heart rate (HR).

Spectral analysis of HRV was calculated using the algorithm of fast Fourier transform according to the recommendations of the American College of Cardiology and American Heart Association [13]. The power of the four main frequency bands was estimated: high frequency (HF) - 0.15-0.40 Hz, low freguency (LF) - 0.04-0.15 Hz, very low frequency (very low frequency - VLF) - 0.0033-0.04 Hz, as well as total power of the spectrum (TP), the ratio of low frequency components to high frequency (LF / HF) was calculated.

During the day time measuring intervals of BP were 15 minutes, and at night – 30 min. one time per hour (according to the recommendations of the Scientific Committee in the field one day monitoring of BP (DMBP) 1990). According to DMBP the average systolic blood pressure (SBP), mean diastolic blood pressure (DBP), the average level of pulse pressure (PBP) were calculated. These indicators were calculated per day, as well as for the period of sleep and wakefulness. The circadian profile of BP was evaluated by calculating the daily index of SBP and DBP, as well as the magnitude and rate morning blood pressure increase.

Echocardiography examination was carried out for studying of cardiac hemodynamics and determining the contractility of the myocardium to exclude organic pathology. Standard methods

were used on the apparatus "PHILLIPS HD3". Aorta, left atrium, right atrium, interventricular septum, and posterior wall thickness, left ventricle in systole and diastole, and the right ventricle were evaluated. *Left ventricular ejection fraction was determined.* Reference values for were provided by the American Society of Echocardiography in conjunction with the European Association of Cardiovascular Imaging [17].

Statistical analysis

The statistical analysis was conducted using the licensed software package "Statistica 6.1" (StatSoftInc). Comparison between groups was carried out by the independent t-test (for parametric data) or the test of Mann-Whitney (nonparametric test). Data with Gaussian distribution were expressed as mean and standard deviation (M±SD). The data were described by median and quarterly ranges (Me (25%; 75%) when their normal distribution was not validated. the Chi^2 test for the comparison between two percentages and the analysis of variance for the comparison between several means. Differences were considered statistically significant at $p < 0.05$.

Results

Average age of patients - 27,6 ± 2,49 years, disease duration - 10,8 ± 1,46 years, BMI 22,6 ± 0,62 kg / m^2

Patients were divided into 2 groups according to the level HвA1c: group 1 (n=21) with HвA1c ≤ 7,5 % and group 2 (n=22) with HвA1c > 7,5 %.

Table 1. General characteristics of patients' groups.

Indicator	Group 1 n=21	Group 2 n=22	P value
Age,years	31.6±2.01	31.5±1.88	0.994
Duration DM 1 type, years	6.3±2.34	12.4±2.21	0.123
BMI kg/m2	23.0±1.16	22.5±2.21	0.716
HbA1c, %	6.65 (6.5; 6.91)	9.6 (8.8; 11.0)*	0.000
Minimal blood glucose, mmol/l	3.96 (2.2; 4.8)	4.71 (2.2; 6.3)	0.343
Maximal blood glucose, mmol/l	12.61 (7.5; 13.9)	16.54 (9.8; 18.7)	0.023*
Average blood glucose, mmol/l	8.37 (6.8; 9.24)	10.72 (8.5; 12.6)	0.017*
Waist circumference / thigh	0.80 (0.69; 0.88)	0,81 (0.76; 0.85)	0.694
C-peptide, ng/ml	0.41 (0.01; 0.57)	0,02 (0.01; 0.11)*	0.018
The daily dose of insulin, unit	36.0±5.22	40.5±10.75*	0.015
Hypoglycemia %	42.9	49.5	0.904
Albuminuria mg/l	22.8 (21.6; 38.9)	37,3 (25.0; 53.8)*	0.035
GFR (CKD-EPI) ml/min/1,73m2	84.6±3.30	81.1±3.52	0.760
Average SBP mm/mercury column	113.2±4.71	115.2±2.13	0.834
Average DBP mm/mercury column	68.3±3.40	71.0±31.44	0.851
Average PBP mm/mercury column	42.9±2.14	45.0±1.22	0.675

Note: * p <0.05, comparing group 2 with group 1

Table 2. Characteristics of HRV in patients' groups

Indicator	Group 1 n=21	Group 2 n=22	P value
SDNN ms cir.period	107.0 (101.5 ; 122.0)	114.5 (90.5; 123.5)	0.140
RMSSD ms cir.period	25.0 (23.0; 42.0)	29.0 (25,0; 42.0)	0.103
pNN50% cir. period	5.0 (4.0; 18.0)	7.5 (5.0; 12.0)	0.109
TP ms^2 cir. period	9075.0 (6754.0; 12488.0)	7493.5 (4527.0; 10717.0)	0.236
VLFms^2 cir.period	6576.0 (4743.,0; 7873.0)	4640.5 (3015.0; 6320.0)	0.539
LF ms^2 cir. period	2167.0 (1669.0; 3357.0)	2138.0 (1192.0; 3544.0)	0.125
HFms^2 cir. period	381.0 (320.0; 1122.0)	627,0 (324.0; 1077.0)	0.147
LF/HF cir. period	4.5 (3.1; 6.7)	4,1 (3.3; 4.9)	0.203

There were no statistical differences between groups in age, duration of disease, the basic anthropometric indexes, daily dose of insulin, the level of daily BP, the GFR level, and

did not differ by the frequency of hypoglycemia, (Table 1), as well as on spectral and temporal characteristics of HRV (Table 2).

The correlation analysis for the duration of Type 1 DM revealed increasing LF / HF (r=0.383; p=0.045), increasing SBP at night (r=0.372; p=0.039) and for 24 hours (r=0.386; p=0.046). Indicators of DBP to the greater extent changed the course of the disease. The direct positive correlation between the duration of Type 1 DM and the increase in the average DBP for 24 hours (r=0.446; p=0.025), mean DBP during the day (r=0.396; p=0.021), mean DBP at night (r=0.537; p=0.020) were revealed, too (Figure 1). Also, this positive correlation was revealed in increasing of daily performance of the diastolic pressure area index (DPAI24) (r=0.422; p=0.028), area index of night DBP (r=0.414; p=0.032), and daily performance of the diastolic pressure time index (DPTI24) (r=0.411; p=0.033), time index of night DBP (r=0.428; p=0.026).

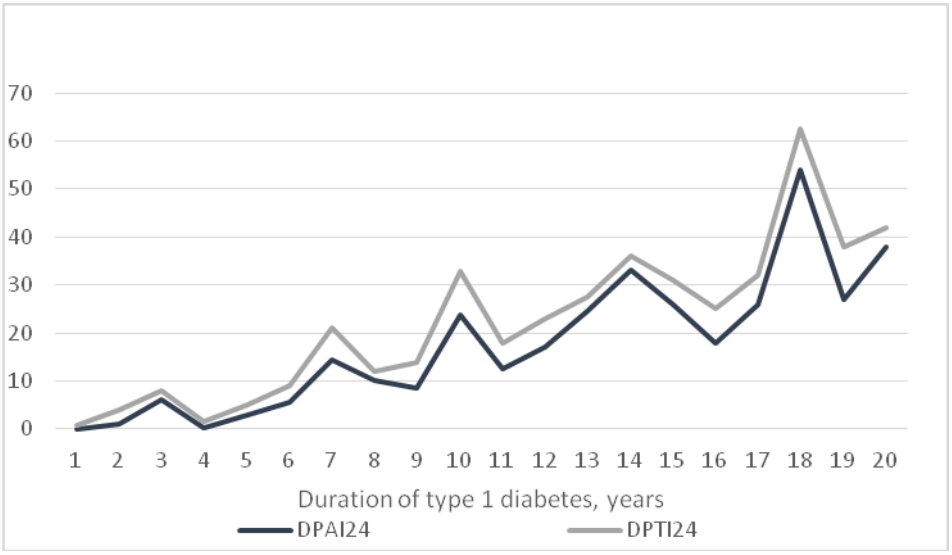

Figure 1. *Dynamics of indicators of DPAI24 and DPTI24 with increasing duration of type 1 diabetes.*

Additionally, the increase in the duration of the disease was accompanied by deterioration in glycemic control, assessed by HbA1c level (r=0.381; p=0.046).

The presence of hypoglycemic episodes affected both on the temporal and spectral HRV indices, irrespective of the level of HbA1c (Table 3). The frequency of hypoglycemic episodes increased with increasing duration of Type 1 DM (r=0.4; p=0.026).

Table 3. Correlation of HRV parameters, depending on hypoglycemia

Indicator	Spearman R	P value
SDNN ms circadian period	-0.339	0.144
SDNN ms night	-0.352	0.061
RMSSD ms circadian period	-0.531*	0.003
RMSSD ms day	-0.373*	0.046
RMSSD ms night	-0.506*	0.005
pNN50% circadian period	-0.503*	0.005
pNN50% day	-0.316*	0.094
pNN50% night	-0.515*	0.004
TP ms^2 night	-0.390*	0.017
LF ms^2 night	-0.406*	0.029
HF ms^2 circadian period	-0.489*	0.007
HF ms^2 day	-0.323*	0.087
HF ms^2 night	-0.506*	0.005
LF/HF circadian period	0.552*	0.002
LF/HF day	0.328*	0.003
LF/HF night	0.522*	0.004

Notice : * p <0,05.

The groups were divided into subgroups according to the absence (A) or presence (B) of hypoglycemia (Table 4).

Table 4. Characteristics of patients' subgroups.

Indicator	Group 1A n=10	Group 1B n=11	P value	Group 2A n=10	Group 2B n=12	P value
Duration DM 1 type, years	2.0±0.52	11.0±3.18*	0.04	9.3±2.26	15.8±4.24	0.144
HbA1c, %	6.62 (6.8; 6.84)	6.61 (6.5; 6.91)	0.981	11.29 (9.1; 10.7)	11.31 (8.8; 11.0)	0.985
C-peptide, ng/ml	0.171±0.07	0.024±0.09 *	0.017	0.16±0.05	0.10±0.01	0.459
Average blood glucose, mmol/l	8.41 (6.8; 9.24)	8.29 (6.5; 9.22)	0.412	10.72 (8.9; 12.2)	10.13 (8.1; 13.4)	0.357
The daily dose of insulin, unit	29.5±5.17	44.7±9.34	0.116	49.5±10.21	43.0±8.54	0.394
SDNN ms cir. period	106.0 (102.5; 122.5)	114.0 (91.5; 123.0)	0.0267	112.5 (96.5; 130.5)	102.0 (77.0; 118.0)	0.167
RMSSD ms cir. period	24.0 (22.0; 41.0)	29,5 (25.5; 42.0)*	0.028	31.5 (28.0; 43.0)	23.5 (14.0; 34.0)*	0.015
pNN50% cir. period	4.5 (4.0; 19.0)	7.0 (5.5; 12.5)*	0.032	8.5 (6.0; 13.5)	4,5 (1.0; 11.0)*	0.024
TP ms^2 cir. period	11220.5 (8356.5; 12781.0)	7305.0 (4225.0; 9057.0)	0.099	8032.0 (6182.0; 10345.0)	6038.0 (3890.0; 11695.0)	0.593
LF ms^2 cir. period	3015.5 (2267.0; 3405.0)	1705.0 (1185.0; 2245.0)	0.081	2459.5 (1895.5; 3604.4)	1526.0 (1030.0; 2930.0)	0.492
HF ms^2 cir. period	1059.5 (332.0; 2118.0)	325.0 (168.0; 830.5)*	0.019	729.0 (495.0; 1153.0)	380.0 (149.0; 713.0)*	0.045
LF/HF cir. period	3.10 (2.10; 4.20)	6.80 (4.55; 6.90)*	0.018	3.40 (2.40; 4.30)	4.35 (4.10; 6.50)*	0.013
Average SBP mm/mercury column	112.0±5.87	115.5±4.33	0.767	115.8±3.18	114.4±4.16	0.746
Average DBP mm/mercury column	67.3±6.25	70.2±7.82	0.716	71.8±10.12	70.1±7.04	0.562
Average PBP mm/mercury column	44.8±7.21	46±6.32	0.537	44.4±6.15	45.6±8.43	0.642
SPAI24	0.1 (0; 10.35)	0.24 (0.8; 13.25)	0.254	0.15 (0; 12.45)	0.55 (0; 12.35)	0.324
SPTI24	0.18 (0; 10.74)	0.26 (0.9; 14.57)	0.387	0.3 (0; 7.9)	1.70 (0.1; 11.9)	0.495
DPAI24	4.3 (0;13.9)	11.5 (0; 13.1)*	0.012	5.20 (1.45; 14.35)	6.80 (3.30; 10.6)*	0.016
DPTI24,%	4.4 (0;14.1)	15.5 (0;14.2)*	0.001	5.75 (2.35; 12.85)	6.40 (0.9; 15.4)*	0.025

Note: * p <0.05, comparing subgroup B with group A within one group.

There was a lower C - peptide mean level in group 1B – 0.024±0.09 vs group 1A – 0.171±0.07 (p=0.017) that probably influenced the occurrence of hypoglycaemic episodes in the group with satisfactory control.

Decreasing temporal indices of HRV was identified in group 2B compared to 2A: RMSSD

per day to 23.5 (14.0; 34.0) vs. 31.5 (28.0; 43.0) (p =0.015), pNN50% per day 4.50 (1.0, 11.0) vs 8.50 (6,00; 13.50) (p =0.024) and daily frequency characteristics of HRV: HF 380.0 (149.0; 713.0) against 729.0 (495.0; 1153.0) (p =0.045). The index LF / HF was significantly lower: 3.40 (2.40; 4.30) vs 4.35 (4.10; 6.50) (p=0.013) in group 2A (Figure 2).

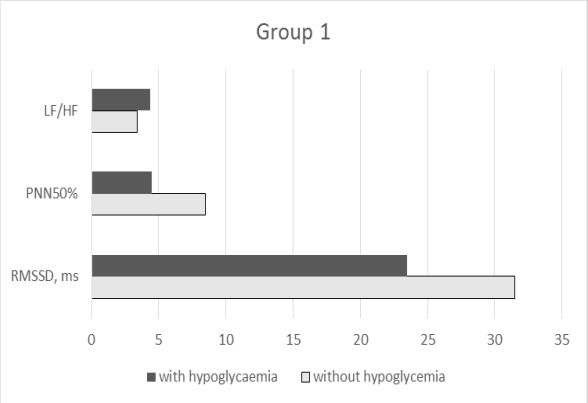

Figure 2. *Indices of HRV in groups with different glycemic control depending on the presence of hypoglycemia.*

Among all BP monitoring parameters the DPAI24 and DPTI24 are the most validative for confirmation of hypoglycemic episodes. In our study was showed DPAI24 6.80,0 (3.30; 10.6) vs 5.20 (1.45; 14.35) (p=0.016), and DPTI24 6.4 (0.9; 15.4) as against 5.75 (2.35; 12.85) (p=0.025), compareing groups 2B and 2A. Comparable data were obtained in group 1: DPAI24 11.5 (0; 13.1) vs 4.3 (0;13.9) (p=0.012), and DPTI24 15.5 (0;14.2) as against 4.4 (0;14.1) (p=0.001), compareing groups 1B and 1A.

Discussion

We confirmed in our study results showed da Silva A.K. et al., 2017, found that patients with type 1 diabetes had a significant decrease in HRV time values, such as RMSSD, PNN50% [18]. In another study da Silva T.P. et al., 2017, studied the spectral analysis of HRV in patients with Type 1 DM. Patients with moderate and severe hypoglycemia were older, had a longer duration of diabetes, which correlated with the astonishment of chronic complications. The authors observed a lower HRV in the high-frequency spectral analysis band, and an increase in the LF / HF index [19].

We showed that hypoglycemic conditions lead to further progression of autonomic dysfunction and even greater reduction of HRV, namely, RMSSD, pNN50%, and an increase in the LF / HF index. The increase of duration Type 1 DM was accompanied by deterioration in glycemic control, assessed by HbA1c level (r = 0.381; p = 0.046) and increased the frequency of hypoglycemia (r = 0.4; p =0.046). Similar results were obtained by Limberg J.K. et al. 2015, which showed that these rates decreased even more during episodes of hypoglycemia [20]. All this changes leads to cardiovascular death.

Although the duration of disease leads to an increase in SBP (r=0.386; p=0.046) and DBP (r=0.446; p=0.025), but the growth of DPAI24 and DPTI24 correlated with the presence of a hypoglycaemic episodes.

Thus, hypoglycemia has negatively affects in cardiovascular functiob both by reducing the temporal and spectral parameters of HRV, and at the expense of increasing DBP, which in turn further reduces the autonomic cardiac activity.

Conclusions

The determination of RMSSD, pNN50%, LF, HF and LF / HF is the most reliable criteria for the evaluation of changes of heart rate variability in patients with Type 1 DM.

The increase the duration of type 1 diabetes has the correlation with the decrease of HRV as by time and frequency indicators, namely, the decrease in RMSSD, pNN50% and the increase of LF/HF are observed.

Hypoglycemia is the key factor leading to the decline in HRV indices, namely RMSSD, pNN50% and increased LF/HF. The incidence of hypoglycaemia episodes increases with duration

of type 1 diabetes, regardless of HbA1c indicator.

The daily variability of BP in patients with type 1 diabetes is realized through the changes of DBP. The duration of the disease has the significant correlation with the increase in the average DBP during the day and in periods of sleep and wakefulness, increased DPAI24.

All the above findings demonstrate the need to reduce the frequency of hypoglycaemic episodes in patients with T1DM, as its impact on HRV and BP, regardless of glycaemia control and duration of disease affects the functional state of the cardiovascular system in a negative mode.

Conflicts of interest: The authors declare that they have no conflict of interest.

REFERENCES

1. International Diabetes Federation. IDF Diabetes Atlas 7th Edition 2015. Available at: https://www.idf.org/e-library/epidemiology-research/diabetes-atlas/13-diabetes-atlas-seventh-edition.html.

2. Martínez-Cerón E, García-Río F. Mortality and cardiovascular disease in type 1 and type 2 diabetes. *N Engl J Med* 377: 300, 2017.

3. Borschuk AP, Everhart RS. Health disparities among youth with type 1 diabetes: A systematic review of the current literature. *Fam Syst Health* 33: 297–313, 2015

4. Rawshani A, Rawshani A, Franzén S, **et al**. Range of Risk Factor Levels: Control, Mortality, and Cardiovascular Outcomes in Type 1 Diabetes Mellitus. *Circulation* 135: 1522-1531, 2017.

5. Eckel RH, Hokanson JE. The Prediction of Atherosclerotic Cardiovascular Disease in Type 1 Diabetes Mellitus: Do We Just Stop Here? *Circulation* 133: 1051-1053, 2016

6. Rosano GM, Vitale C, Seferovic P. Heart Failure in Patients with Diabetes Mellitus. *Card Fail Rev* 3: 52–55, 2017.

7. American Diabetes Association. Standards of medical care in diabetes - 2017. *Diabetes Care* 40: 11-104, 2017

8. Turker Y, Aslantas Y, Aydin Y, et al. Heart rate variability and heart rate recovery in patients with type 1 diabetes mellitus. *Acta Cardiol* 68: 145-150, 2013.

9. Galli-Tsinopoulou A, Chatzidimitriou A, Kyrgios I, Rousso I, Varlamis G, Karavanaki K. Children and adolescents with type 1 diabetes mellitus have a sixfold greater risk for prolonged QTc interval. *Pediatr Endocrinol Metab* 27: 237-243, 2014.

10. Gruden G, Giunti S, Barutta F, et.al. QTc interval prolongation is independently associated with severe hypoglycemic attacks in type 1 diabetes from the EURODIAB IDDM complications study. *Diabetes Care* 35: 125-127, 2012.

11. Koivikko ML, Tulppo MP, Kiviniemi AM, et al. Autonomic cardiac regulation during spontaneous nocturnal hypoglycemia in patients with type 1 diabetes. *Diabetes C*are 35: 1585-1590, 2012.

12. Jaiswal M, Urbina EM,Wadwa RP et al. Reduced heart rate variability among youth with type 1 diabetes: the SEARCH CVD study. *Diabetes Care* 36(1): 157–162, 2013.

13. Task Force of the European Society of Cardiology and the American Society of Pacing Electrophysiology. Heart rate variability: standards of measurement, physiological interpretation, and clinical use. *Circulation* 93: 1043–1065, 1996.

14. Baumert M, Porta A, Vos MA, et al. QT interval variability in body surface ECG: measurement, physiological basis, and clinical value: position statement and consensus guidance endorsed by the European Heart Rhythm Association jointly with the ESC Working Group on Cardiac Cellular Electrophysiology. *Europace* 18: 925-944, 2016.

15. National Kidney Foundation. KDIGO 2012 clinical practice guideline for the evaluation and

management of chronic kidney disease. *Kidney Int* 3: 40-47, 2013.

16. iPro2 CGM User Guide. Available at: https://www.medtronicdiabetes.com/sites/default/files/library/download-library/user-guides/iPro2-with-Enlite-User-Guide.pdf.

17. Lang RM, Badano LP, Victor Mor-Avi V, et al. Recommendations for Cardiac Chamber Quantification by Echocardiography in Adults: An Update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. *Am Soc Echocardiogr* 18: 1440-1463, 2005.

18. da Silva AKF, Christofaro DGD, Bernardo AFB, Vanderlei FM, Vanderlei LCM. Sensitivity, Specificity and Predictive Value of Heart Rate Variability Indices in Type 1 Diabetes Mellitus. *Arq Bras Cardiol* 108: 255-262, 2017.

19. da Silva TP, Rolim LC, Sallum FC, Zimmermann LM, Malerbi F, Dib SA. Association between severity of hypoglycemia and loss of heart rate variability in patients with type 1 diabetes mellitus. *Diabetes Metab Res Rev* 33, 2017 (Abstract).

20. Limberg JK, Dube S, Kuijpers M, et al. Effect of hypoxia on heart rate variability and baroreflex sensitivity during hypoglycemia in type 1 diabetes mellitus. *Clin Auton Res* 25: 243-250, 2015.